Attorney Docket No.: 10527.204-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Nielsen *et al.* Confirmation No: 1622

Serial No.: 10/582,277 Group Art Unit: 1656

Filed: June 10, 2006 Examiner: S. Noakes

For: A Cell with Improved Secretion Mediated by MrgA Protein or Homologue

APPEAL BRIEF UNDER 37 C.F.R. §41.37

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Having filed a Notice of Appeal from the rejection of claims 41 and 46-65, the final rejection being mailed on 20 May 2009, Appellant submits its Appeal Brief for the above-captioned application pursuant to 37 C.F.R. §41.37. Accordingly, Appellant hereby appeals the final rejection of claims 41 and 46-65, all the claims pending in the present application.

(I) Real Party In Interest

The real party in interest is Novozymes A/S.

(II) Related Appeals and Interferences

There are no related appeals, interferences or judicial proceedings known to Appellant, the Appellant's legal representative, or Assignee which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(III) Status of the Claims

Claims 1-40, 42-45 are canceled. Claims 41 and 46-65 remain pending in the application. Claims 41 and 46-65 stand rejected and are the subject of the instant appeal. All pending claims (a copy of which is included in the Claims Appendix), are included in this appeal.

(IV) Status of Amendments

The response filed under 37 C.F.R. § 1.116 on 19 October 2009 was entered, and overcame the rejection made under 35 U.S.C. 112 1st paragraph-enablement, but did not overcome the final rejection of claims 41 and 46-65 under 35 U.S.C. § 102(b).

(V) Summary of Claimed Subject Matter

Independent Claim 41

The claimed invention, as presented in Claim 41, the first independent claim, is directed to a method for enhancing secretion of a protein of interest, the method including expressing the protein in a *Bacillus* progeny cell derived from a *Bacillus* parent cell, wherein

a) the *Bacillus* progeny cell includes at least one gene encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 and, optionally, further comprising a DNA segment operably linked with the encoding gene, wherein the gene and, optionally the DNA segment is manipulated with respect to the parent cell; or

b) the *Bacillus* progeny cell comprises two or more copies of a gene encoding MrgA protein with an amino acid sequence which has at least 90% identity to the amino acid sequence shown in SEQ ID NO:2, wherein the *Bacillus* progeny cell produces greater amounts of MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 than the parent cell, and wherein the *Bacillus* progeny cell produces greater amounts of protein of interest than the *Bacillus* parent cell.

As disclosed in the specification at page 2, lines 11-20:

> Accordingly, in a first aspect the invention relates to a progeny cell derived from a parent cell, wherein
> a) the progeny cell comprises at least one gene encoding MrgA protein or a functional homologue thereof and/or a DNA segment operably linked with the encoding gene, wherein said gene and/or DNA segment is manipulated with respect to the parent cell;
> b) the progeny cell comprises two or more copies of a gene encoding MrgA protein or a functional homologue thereof; or
> c) the progeny cell is mutated with respect to the parent cell;
> whereby the progeny cell produces greater amounts of MrgA protein or a functional homologue thereof than the parent cell.

As disclosed in the specification at page 2, lines 28-30:

> A second aspect of the invention relates to a method for enhancing secretion of a protein of interest, the method comprising expressing said protein in a cell according to the first aspect.

As disclosed in the specification at page 14, lines 15-29:

> The MrgA protein or functional homologue thereof may be a wild-type protein identified and isolated from a natural source. Such wild-type proteins may be specifically screened for by standard techniques known in the art. Furthermore, the MrgA protein or functional homologue thereof may be prepared by the DNA shuffling technique, such as described in J.E. Ness et al. Nature Biotechnology 17, 893-896 (1999). Moreover, the MrgA protein or functional homologue thereof may be an artificial variant. Such artificial variants may be constructed by standard techniques known in the art, such as by site-directed/random mutagenesis. In one embodiment of the invention, amino acid changes (in the artificial variant as well as in wild-type polypeptides) are of a minor nature, that is conservative amino acid substitutions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to about 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

As disclosed in the specification at page 16, lines 9-20:

> The cell of the invention produces greater amounts of MrgA protein or a functional homologue thereof, than the parent cell. A comparison should be made by cultivating the cell of the invention as well as the parent cell under essentially identical conditions, and comparing the amount of MrgA protein by any standard method in the art. Preferably the cell of the invention produces at least 5% more MrgA than the parent, more preferably at least 10%, still more preferably at least 20%, and most preferably at least 50% more MrgA protein or a functional homologue thereof than the parent. Such overproduction may be accomplished by standard means known to the art, *e.g.,* use of multicopy plasmids, multiple copies of the genes encoding MrgA or a functional homologue thereof, and/or the protein of interest, in the chromosome of the host, combined with altering the regulatory elements to increase expression, *e.g.,* use of strong promoter(s), use of multiple promoters, use of enhancers, and so forth.

As disclosed on page 16, lines 21-23.

> As the inventors show herein, a cell of the first aspect is capable of producing greater amounts of a protein of interest than the corresponding parent cell, when both are cultivated under essentially identical conditions.

As disclosed in the specification at page 17, lines 13-15:

> A preferred embodiment relates to the cell of the invention, which is a bacterial cell, preferably a prokaryotic cell, more preferably a Gram-positive cell, and most preferably of the genus *Bacillus* . . .

As disclosed in the specification at page 17, lines 31-35:

> Specifically, a preferred embodiment relates to the cell, wherein the MrgA protein or functional homologue thereof comprises an amino acid sequence which is at least 70% identical to the amino acid sequence shown in SEQ ID NO:2, preferably at least 75%, 80%, 85%, 90%, 95%, 97%, or even 99% identical to the amino acid sequence shown in SEQ ID NO:2.

Independent Claim 46

The claimed invention, as presented in Claim 46, the second independent claim, is directed to a method for producing a protein of interest, including the steps of: a) cultivating a *Bacillus* progeny cell derived from a *Bacillus* parent cell, wherein

1) the *Bacillus* progeny cell comprises at least one gene encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 and, optionally, further comprising a DNA segment operably linked with the encoding gene, wherein the gene and, optionally the DNA segment is manipulated with respect to the *Bacillus* parent cell; or

2) the *Bacillus* progeny cell comprises two or more copies of a gene encoding MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2, wherein the *Bacillus* progeny cell produces greater amounts of MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 than the *Bacillus* parent cell, and wherein the *Bacillus* progeny cell produces greater amounts of a protein of interest than the *Bacillus* parent cell; and

b) recovering the protein.

As disclosed in the specification at page 2, lines 11-20:

> Accordingly, in a first aspect the invention relates to a progeny cell derived from a parent cell, wherein
>
> a) the progeny cell comprises at least one gene encoding MrgA protein or a functional homologue thereof and/or a DNA segment operably linked with

the encoding gene, wherein said gene and/or DNA segment is manipulated with respect to the parent cell;

b) the progeny cell comprises two or more copies of a gene encoding MrgA protein or a functional homologue thereof; or

c) the progeny cell is mutated with respect to the parent cell;

whereby the progeny cell produces greater amounts of MrgA protein or a functional homologue thereof than the parent cell.

As disclosed in the specification at page 2, lines 35-73 to page 3 lines 1:

> In a fourth aspect the invention relates to a method for producing a protein of interest, comprising the steps of:
> a) cultivating a cell as defined in the first aspect; and
> b) recovering the protein.

As disclosed in the specification at page 14, lines 15-29:

> The MrgA protein or functional homologue thereof may be a wild-type protein identified and isolated from a natural source. Such wild-type proteins may be specifically screened for by standard techniques known in the art. Furthermore, the MrgA protein or functional homologue thereof may be prepared by the DNA shuffling technique, such as described in J.E. Ness et al. Nature Biotechnology 17, 893-896 (1999). Moreover, the MrgA protein or functional homologue thereof may be an artificial variant. Such artificial variants may be constructed by standard techniques known in the art, such as by site-directed/random mutagenesis. In one embodiment of the invention, amino acid changes (in the artificial variant as well as in wild-type polypeptides) are of a minor nature, that is conservative amino acid substitutions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to about 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

As disclosed in the specification at page 16, lines 9-20:

> The cell of the invention produces greater amounts of MrgA protein or a functional homologue thereof, than the parent cell. A comparison should be made by cultivating the cell of the invention as well as the parent cell under essentially identical conditions, and comparing the amount of MrgA protein by any standard method in the art. Preferably the cell of the invention produces at least 5% more MrgA than the parent, more preferably at least 10%, still more preferably at least 20%, and most preferably at least 50% more MrgA protein or a functional homologue thereof than the parent. Such overproduction may be accomplished by standard means known to the art, *e.g.,* use of multicopy plasmids, multiple copies of the genes encoding MrgA or a functional homologue thereof, and/or the protein of interest, in the chromosome of the host, combined with altering the regulatory elements to increase expression, *e.g.,* use of strong promoter(s), use of multiple promoters, use of enhancers, and so forth.

As disclosed on page 16, lines 21-23.

> As the inventors show herein, a cell of the first aspect is capable of producing greater amounts of a protein of interest than the corresponding parent cell, when both are cultivated under essentially identical conditions.

As disclosed in the specification at page 17, lines 13-15:

> A preferred embodiment relates to the cell of the invention, which is a bacterial cell, preferably a prokaryotic cell, more preferably a Gram-positive cell, and most preferably of the genus *Bacillus* . . .

As disclosed in the specification at page 17, lines 31-35:

> Specifically, a preferred embodiment relates to the cell, wherein the MrgA protein or functional homologue thereof comprises an amino acid sequence which is at least 70% identical to the amino acid sequence shown in SEQ ID NO:2, preferably at least 75%, 80%, 85%, 90%, 95%, 97%, or even 99% identical to the amino acid sequence shown in SEQ ID NO:2.

(VI) Grounds of Rejection to be Reviewed on Appeal

The grounds of rejection to be reviewed on appeal are:

(1) Whether the Chen reference anticipates Claims 41 and 46-65 pursuant to 35 U.S.C. § 102(b).

(VII) ARGUMENT

A. Chen does not anticipate Claims 41 and 46-65 under 35 U.S.C. 102(b).

1. The Cited Reference

Chen *et al. Bacillus subtilis MrgA is a DPS(PexB) homologue: evidence for metalloregulation of an oxidative-stress gene*; *Molecular Microbiology*, 18(2), 295-300 (1995) (hereinafter referred to simply as "Chen").

2. The Rejection under 35 U.S.C. 102(b)

The Examiner's anticipation rejection is reproduced below from the Final Office Action dated 20 May 2009:

> Claims 41 and 46-65 are rejected under 35 U.S.C. 102(b) as being anticipated by Chen et al. (Mol. Micro., 1995, cited on IDS).
>
> Chen et al. teach a strain of parent cells known as MA991 which are hydrogen peroxide sensitive cells that constitutively express catalase (KatA) and alkyl hydrogen peroxide reductase (AhpC and AhpF), as well as two other proteins of 113 kDA and 16kDa. Chen et al. transformed these cells (MA991) by

inserting the heterologous genes mrgA-lacZ to express said MrgA and beta-galactosidase proteins, thus resulting in progeny cells (Strain HB1032 - a Bacillus subtilis strain - meets claims 47-48 and 59-60) - See p. 297, 1st column, paragraphs 1-2. It was noted that N-terminal sequencing indicated that the 113 kDa and 16kDa protein over expressed in MA991 and thus HB 1032 was identical to, and thus, MrgA (the former being in oligormeric complex the later a monomer - See p. 297, 1st column, paragraph 2) and the sequence for the MrgA is shown in Figure 1, it is noted [*sic*] said sequence is 100% identical to the instant SEQ ID NO:2 (thus, meets claims 41, 46, 51-58, 63-65. It is further noted that lacZ, e.g. a protein of interest, produces a beta-galactosidase [sic] (e.g. meets claims 50 and 62). Thus, strain HB 1032 possesses at least two copies of MrgA (e.g. one naturally occurring in the parent strain, the other having been introduced into the progeny cell). Simple induction of the MrgA-LacZ fusion creates a progeny cell that produces significantly more MrgA and LacZ than the parent strain. Thus, this meets the limitations of the claims.

Examiners Arguments and Examiner's Rebuttal:

Applicants traverse the rejection and state: Nowhere does Chen describe progeny cells that produce greater amounts of the claimed MrgA than the parent cell AND greater amounts of the protein of interest than the parent cell.

The Examiner, however, disagrees. The progeny cell has been designed to overproduce an MrgA-LacZ fusion protein. Thus, the protein of interest is LacZ. The simple induction and over expression of this fusion construct necessarily results in an overproduction of BOTH the LacZ and the MrgA proteins (see p. 296, wherein MrgA-lacZ expression was induced 6-10 fold). Thus, this meets the limitations of the claims. Since it is known and even stated in the specification that *Bacillus subtilis* inherently produces MrgA, then inserting the MrgA-LacZ fusion construct necessarily and inherently means that the progeny cell has at least one copy of said MrgA gene being over produced and even has two copies of said gene. It is further noted that the sequence listed in Figure 1 for the MrgA protein is identical to SEQ ID NO:2.

The Advisory Action dated 3 November 2009 maintained the rejection and informed:

Continuation of 11, does NOT place the application in condition for allowance because: The Examiner acknowledges the amendments filed 19 October 2009 which materially reduces the number of rejections and objections and thus said amendments are entered. Said amendments overcome the previous Enablement rejection and all other objections of record. Thus, the only remaining rejection of record is the rejection of claims 41 and 46-65 under 35 U.S.C. 102(b), Chen et al. (as cited on the IDS). It is Applicants position that the Examiner has interpreted the reference inaccurately in the following way: Applicants cited the passage on p. 297 from Chen et al. which states the introduction of the mrgA-lacZ fusion protein abolishes MrgA protein production in progeny HB1032 as is evidenced by the SDS-PAGE gel in Fig. 4. It is further stated that the HB1032 progeny strain does not contain a single copy of the MrgA gene and "is incapable of producing MrgA protein." (see Remarks filed 10/19/09, p.6-7). And finally Chen et al. does not teach that progeny cells produce more amounts of MrgA than the parent strain.

The Examiner, however, disagrees with these assertions and maintains her interpretation of Chen et al. is accurate. The passage that Applicants cite and their interpretation is noted, however, the Examiner interprets that this merely means that the insertion of the MrgA-LacZ fusion protein, which Applicants also note and state, has been inserted into the chromosomal MrgA gene and thus disrupts the MrgA gene which exists in the parent strain. As such this abolishes the progeny strains (HB1032) ability to produce the chromosomal version of the MrgA protein; thus the lack of the 16kDa and 113kDa bands as shown in Figure 4. However, the fusion construct MrgA-LacZ inserted into the progeny cells/strain certainly exists and still produces a fully functional MrgA:LacZ fusion proteins required by the claim. Furthermore, when one looks at strain HB 1022, and Figure 3, induction of the fusion protein MrgA protein-LacZ similarly in this strain produces 6-10 times more protein than without induction and thus more protein of interest (LacZ) and MrgA protein is produces as compared to the parent strain (See full last paragraph, p. 296 and Figure 3, p. 297). Recovery of protein of interest would have been included in the B-gal assays wherein said assay was performed by lysing the cells, using the supernatant and testing for the beta-galactosidase activity.

3. The Legal Standard

For a reference to anticipate a claim under 35 U.S.C. § 102, each and every claim element must be found in the cited reference. *Verdegaal Brothers, Inc. v. Union Oil Co. of California*, 814 F.2d 628, 2 USPQ2d 1051 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989). The elements must be arranged as required by the claim, but this is not an *ipsissimis verbis* test, *i.e.*, identity of terminology is not required. *In re Bond*, 910 F.2d 831, 15 USPQ2d 1566 (Fed. Cir. 1990).

4. The Rejection under 35 U.S.C. 102(b) over Chen.

(a) Chen fails to teach all elements of Claim 41, thus Claim 41 is not anticipated by Chen.

Independent Claim 41 refers to a method for enhancing secretion of a protein of interest, the method including expressing the protein in a *Bacillus* progeny cell derived from a *Bacillus* parent cell, which requires, *inter alia*, the *Bacillus* progeny cell include at least one gene encoding or two or more copies of a gene encoding MrgA protein with an amino acid sequence which has at least 90% identity to the amino acid sequence shown in SEQ ID NO:2, wherein the *Bacillus* progeny cell produces greater amounts of MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 than the parent cell, and wherein the *Bacillus* progeny cell produces greater amounts of protein of interest than the *Bacillus* parent cell. In other words, the *Bacillus* progeny cell must include: 1) one or at least two genes

encoding MrgA protein with an amino acid sequence which has at least 90% identity to the amino acid sequence shown in SEQ ID NO:2; and 2) produce greater amounts of MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 than the parent cell.

Contrary to the Examiner's assertion, the progeny cells of Chen (HB1032) are devoid of a gene encoding MrgA protein with an amino acid sequence which has at least 90% identity to the amino acid sequence shown in SEQ ID NO:2. Chen *et al.* clearly states on page 297:

> To test the relationship between *mrgA* and this 113 kDa protein, we transformed our *mrgA-lacZ* fusion into MA991 to generate strain HB1032. In stationary phase, HB1032 still overproduced KatA, AhpC and AhpF, but neither the 16 kDa nor the 113 kDa protein was observed by SDS-PAGE (Fig. 4). This suggests that *mrgA* is the structural gene for these two observed protein bands; the 113 kDa protein band presumably represents a stable oligomeric complex while the 16 kDa band is the appropriate size for the MrgA monomer.

Accordingly, the structural gene was changed and the 16KDa protein (mrgA monomer) was not made in the progeny cell (HB1032). Here, the introduction of the *mrgA-lacZ* fusion into strain MA991 completely abolished production of MrgA protein in the resulting HB1032 progeny strain as evidenced by the SDS-PAGE gel in figure 4. (*See* FIG. 4). Thus, there is no gene in accordance with Claim 41 in the Chen reference.

Further, strain HB1032 does not include an intact copy of the *mrgA* gene and is incapable of producing MrgA protein. Strain HB1032 appears to be constructed by site-specific integration of a *lacZ*-reporter into the chromosomal *mrgA* gene of MA991. *See*, *e.g.*, the figure legend of FIG. 4 where the genotype of HB1032 is shown as (MA991 *mrgA*::Tn*917-lacZ*) indicating to one skilled in the art that the genomic *mrgA* gene had been interrupted by the Tn*917-lacZ* insert. Accordingly, strain HB1032 does not include one single intact copy of the *mrgA* gene and is incapable of producing MrgA protein. This is also true for strain HB 1022 (mrgA::Tn917-lacZ) which was used in Chen to demonstrate that disruption of the MrgA. See Chen page 296, Column 2. Thus, nowhere does Chen describe a progeny *Bacillus* cell including at least one or two or more genes encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2.

Contrary to the Examiner's assumptions, Chen does not show a progeny *Bacillus* cell producing greater amounts of MrgA than the parent *Bacillus* cell. In the advisor action, the Examiner explained:

> the Examiner interprets that this merely means that the insertion of the MrgA-LacZ fusion protein . . . has been inserted into the chromosomal MrgA gene and

> thus disrupts the MrgA gene which exists in the parent strain. As such this abolishes the progeny strains (HB1032) ability to produce the chromosomal version of the MrgA protein . . .

Thus, the Examiner appears to have acknowledged that the progeny HB1032 strain does not produce MrgA protein, however the Examiner concludes, without any scientific rationale, that "the fusion construct MrgA-LacZ inserted into the progeny cells/strain certainly exists and still produces a fully functional MrgA:LacZ fusion proteins required by the claim." See Advisory Action dated 3 November 2009. There is no basis in Chen to conclude that the Mrga:LacZ fusion protein is fully functional and one of ordinary skill in the art would not conclude that the fusion protein is fully functional with respect to the MrgA and the LacZ --independently. Rather, one of ordinary skill in the art would read the genotype of strain HB 1032 in figure 4 (MA991 mrgA::Tn917-lacZ) and HB 1022 (Fig. 2) and conclude that the mrgA gene has been interrupted by the insertion of a Tn917 transposon including a promoterless LacZ reporter gene. Accordingly, the genotype indicated by (mrgA::Tn917-lacZ) means that the mrgA gene-product is truncated and fused to the lacZ enzyme after the point of truncation. The MrgA protein of *Bacillus subtilis* consists of 153 amino acids (See Seq ID No:2) so the Examiner has incorrectly presupposed without any evidence or scientific rationale that a fully functional MrgA-LacZ fusion protein as required by the claim is produced. The only apparent functional part of the MrgA-LacZ fusion protein of Chen is the beta-galactosidase activity of the LacZ portion. The MrgA protein required by independent claim 41 is absent in Chen.

In any event, it is in appropriate for the Examiner to refer to one MrgA:LacZ fusion product as both the MrgA protein and protein-of-interest in order to satisfy all elements. In Chen, the fusion product production in HB 1022 is increased 6 to 10 fold by an inducible response to H_2O_2. The invention of the present disclosure enhances secretion of a protein of interest by, *inter alia*, including at least one gene encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 in the progeny cell. In this case it is clear that the Chen does not show the identical invention in as complete detail as Claim 41.

For the foregoing reasons, Appellants submit that the Examiner has erred and Chen fails to anticipate claim 41.

(b) Chen fails to teach all elements of Claim 46, thus Claim 46 is not anticipated by Chen.

Independent Claim 46 refers to a method for producing a protein of interest, including the steps of: a) cultivating a *Bacillus* progeny cell derived from a *Bacillus* parent cell, wherein

1) the *Bacillus* progeny cell includes at least one gene encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 and, optionally, further comprising a DNA segment operably linked with the encoding gene, wherein said gene and, optionally said DNA segment is manipulated with respect to the *Bacillus* parent cell; or

2) the *Bacillus* progeny cell comprises two or more copies of a gene encoding MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2, wherein the *Bacillus* progeny cell produces greater amounts of MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 than the *Bacillus* parent cell, and wherein the *Bacillus* progeny cell produces greater amounts of a protein of interest than the *Bacillus* parent cell; and

b) recovering the protein.

Contrary to the Examiner's assertion, the progeny cells of Chen (HB1032) are devoid of a gene encoding MrgA protein with an amino acid sequence which has at least 90% identity to the amino acid sequence shown in SEQ ID NO:2. Chen *et al.* clearly states on page 297:

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Accordingly, the structural gene was changed and the 16KDa protein (MrgA monomer) was not made in the progeny cell (HB1032). Here, the introduction of the *mrgA-lacZ* fusion into strain MA991 completely abolished production of MrgA protein in the resulting HB1032 progeny strain as evidenced by the SDS-PAGE gel in figure 4. (*See* FIG. 4). Thus, there is no gene in accordance with Claim 41 in the Chen reference.

Further, strain HB1032 does not include an intact copy of the *mrgA* gene and is incapable of producing MrgA protein. Strain HB1032 appears to be constructed by site-specific

integration of a *lacZ*-reporter into the chromosomal *MrgA* gene of MA991. *See*, *e.g.*, the figure legend of FIG. 4 where the genotype of HB1032 is shown as (MA991 *mrgA*::Tn*917-lacZ*) indicating to one skilled in the art that the genomic *mrgA* gene had been interrupted by the Tn*917-lacZ* insert. Accordingly, strain HB1032 does not include one single intact copy of the *mrgA* gene and is incapable of producing MrgA protein. This is also true for strain HB 1022 (mrgA::Tn917-lacZ) which was used in Chen to demonstrate that disruption of the MrgA. See Chen page 296, Column 2. Thus, nowhere does Chen describe a progeny *Bacillus* cell including at least one or two or more genes encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2.

Contrary to the Examiner's assumptions, Chen does not show a progeny *Bacillus* cell producing greater amounts of MrgA than the parent *Bacillus* cell. In the advisor action, the Examiner explained:

> the Examiner interprets that this merely means that the insertion of the MrgA-LacZ fusion protein . . . has been inserted into the chromosomal MrgA gene and thus disrupts the MrgA gene which exists in the parent strain. As such this abolishes the progeny strains (HB1032) ability to produce the chromosomal version of the MrgA protein . . .

Thus, the Examiner appears to have acknowledged that the progeny HB1032 strain does not produce MrgA protein, however the Examiner concludes, without any scientific rationale, that "the fusion construct MrgA-LacZ inserted into the progeny cells/strain certainly exists and still produces a fully functional MrgA:LacZ fusion proteins required by the claim." See Advisory Action dated 3 November 2009. There is no basis in Chen to conclude that the Mrga:LacZ fusion protein is fully functional and one of ordinary skill in the art would not conclude that the fusion protein is fully functional with respect to the MrgA and the LacZ --independently. Rather, one of ordinary skill in the art would read the genotype of strain HB 1032 in figure 4 (MA991 mrgA::Tn917-lacZ) and HB 1022 (Fig. 2) and conclude that the mrgA gene has been interrupted by the insertion of a Tn917 transposon including a promoterless LacZ reporter gene. Accordingly, the genotype indicated by (mrgA::Tn917-lacZ) means that the mrgA gene-product is truncated and fused to the lacZ enzyme after the point of truncation. The MrgA protein of *Bacillus subtilis* consists of 153 amino acids (See Seq ID No:2) so the Examiner has incorrectly presupposed without any evidence or scientific rationale that a fully functional MrgA-LacZ fusion protein as required by the claim is produced. The only apparent functional part of the MrgA-LacZ fusion protein of Chen is the beta-galactosidase activity of the LacZ portion. The MrgA protein required by independent claim 46 is absent in Chen.

In any event, it is in appropriate for the Examiner to refer to one MrgA:LacZ fusion product as both the MrgA protein and protein-of-interest in order to satisfy all elements. In Chen, the fusion product production is increased 6 to 10 fold by an inducible response to H_2O_2. The invention of the present disclosure enhances producing a protein of interest by, *inter alia*, including at least one gene encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 in the progeny cell. In this case it is clear that the prior art does not show the identical invention in as complete detail as Claim 46.

For the foregoing reasons, Appellants submit that the Examiner has erred and Chen fails to anticipate claim 46.

(VIII) CLAIMS APPENDIX

A copy of the claims involved in the appeal is provided in the Claims Appendix attached hereto.

(IX) EVIDENCE APPENDIX

Applicants are not relying on any evidence submitted pursuant to 37. C.F.R. 1.130, 1.131, and 1.132 of this title or of any other evidence entered by the examiner and relied upon by the appellant in the appeal.

(X) RELATED PROCEEDINGS APPENDIX

There are no related appeals and interferences pursuant to 37 C.F.R. 41.37(c)(1)(ii).

(XI) CONCLUSION

For the foregoing reasons, Applicants submit that Claims 41 and 46-65 are patentable over the cited prior art. As such, Applicants respectfully request that the rejections of each of Claims 41 and 46-65 be reversed and the Examiner be directed to issue a Notice of Allowance allowing each of Claims 41 and 46-65.

Should any fee be due, the USPTO is authorized to charge the deposit account of Novozymes North America, Inc. Deposit Account No. 50-1701.

Respectfully submitted,

Date: May 19, 2010

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CLAIMS APPENDIX

Copy of Claims Involved in the Appeal

Claim 41 A method for enhancing secretion of a protein of interest, the method comprising expressing said protein in a *Bacillus* progeny cell derived from a *Bacillus* parent cell, wherein

a) the *Bacillus* progeny cell comprises at least one gene encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 and, optionally, further comprising a DNA segment operably linked with the encoding gene, wherein said gene and, optionally said DNA segment is manipulated with respect to the parent cell; or

b) the *Bacillus* progeny cell comprises two or more copies of a gene encoding MrgA protein with an amino acid sequence which has at least 90% identity to the amino acid sequence shown in SEQ ID NO:2,

wherein the *Bacillus* progeny cell produces greater amounts of MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 than the parent cell, and wherein the *Bacillus* progeny cell produces greater amounts of protein of interest than the *Bacillus* parent cell.

Claim 46 A method for producing a protein of interest, comprising the steps of:

a) cultivating a *Bacillus* progeny cell derived from a *Bacillus* parent cell, wherein

1) the *Bacillus* progeny cell comprises at least one gene encoding metallo regulated gene A (MrgA) protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 and, optionally, further comprising a DNA segment operably linked with the encoding gene, wherein said gene and, optionally said DNA segment is manipulated with respect to the *Bacillus* parent cell; or

2) the *Bacillus* progeny cell comprises two or more copies of a gene encoding MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2, wherein the *Bacillus* progeny cell produces greater amounts of MrgA protein with an amino acid sequence having at least 90% identity to the amino acid sequence shown in SEQ ID NO:2 than the *Bacillus* parent cell, and wherein the *Bacillus* progeny cell produces greater amounts of a protein of interest than the *Bacillus* parent cell; and

b) recovering the protein.

Claim 47 A method in accordance with claim 41, wherein the progeny cell is a *Bacillus* bacterial cell.

Claim 48 A method in accordance with claim 41, wherein the *Bacillus* progeny cell is of a species chosen from the group consisting of *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus coagulans, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus stearothermophilus, Bacillus subtilis,* and *Bacillus thuringiensis*.

Claim 49 A method in accordance with claim 41, wherein said protein of interest is homologous or heterologous.

Claim 50 A method in accordance with claim 41, wherein said protein is a protease, a lipase, a cutinase, an amylase, a galactosidase, a pullulanase, a cellulase, a glucose isomerase, a protein disulphide isomerase, a CGT'ase (cyclodextrin gluconotransferase), a phytase, a glucose oxidase, a glucosyl transferase, lactase, bilirubin oxidase, a xylanase, an antigenic microbial or protozoan protein, a bacterial protein toxin, a microbial surface protein, or a viral protein.

Claim 51 A method in accordance with claim 41, wherein the MrgA protein comprises an amino acid sequence which is at least 95% identical to the amino acid sequence shown in SEQ ID NO: 2.

Claim 52 A method in accordance with claim 41, wherein the MrgA protein comprises the amino acid sequence shown in SEQ ID NO: 2.

Claim 53 A method in accordance with claim 41, wherein the *Bacillus* progeny cell comprises at least one exogenous copy of a polynucleotide encoding MrgA protein comprising an amino acid sequence which is at least 95% identical to the amino acid sequence shown in SEQ ID NO: 2.

Claim 54 A method in accordance with claim 41, wherein the *Bacillus* progeny cell comprises at least one exogenous copy of a polynucleotide encoding MrgA protein comprising the amino acid sequence shown in SEQ ID NO: 2.

Claim 55 A method in accordance with claim 41, wherein the *Bacillus* progeny cell comprises at least one exogenous copy of a polynucleotide, which:

a) comprises a polynucleotide sequence which is at least 90% identical to the sequence shown in SEQ ID NO: 1; or

b) hybridizes with the sequence shown in SEQ ID NO: 1, under medium stringency conditions.

Claim 56 A method in accordance with claim 41, wherein the *Bacillus* progeny cell comprises at least one exogenous copy of a gene encoding the MrgA protein transcribed from one or more heterologous and, optionally, artificial promoter.

Claim 57 A method in accordance with claim 41, wherein the *Bacillus* progeny cell comprises at least one exogenous copy of a gene encoding the MrgA protein integrated into the genome of the cell.

Claim 58 A method in accordance with claim 41, wherein the *Bacillus* progeny cell comprises at least one exogenous copy of a gene encoding the MrgA protein present on an extra-chromosomal construct.

Claim 59 A method in accordance with claim 46, wherein the *Bacillus* progeny cell is a bacterial cell.

Claim 60 A method in accordance with claim 46, wherein the *Bacillus* progeny cell is of a species chosen from the group consisting of *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus coagulans, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus stearothermophilus, Bacillus subtilis,* and *Bacillus thuringiensis*.

Claim 61 A method in accordance with claim 46, wherein said protein of interest is homologous or heterologous.

Claim 62 A method in accordance with claim 46, wherein said protein is a protease, a lipase, a cutinase, an amylase, a galactosidase, a pullulanase, a cellulase, a glucose isomerase, a protein disulphide isomerase, a CGT'ase (cyclodextrin gluconotransferase), a phytase, a glucose oxidase,

a glucosyl transferase, lactase, bilirubin oxidase, a xylanase, an antigenic microbial or protozoan protein, a bacterial protein toxin, a microbial surface protein, or a viral protein.

Claim 63 A method in accordance with claim 46, wherein the MrgA protein comprises an amino acid sequence which is at least 95% identical to the amino acid sequence shown in SEQ ID NO: 2.

Claim 64 A method in accordance with claim 46, wherein the MrgA protein or comprises the amino acid sequence shown in SEQ ID NO: 2.

Claim 65 A method in accordance with claim 46, wherein the *Bacillus* progeny cell comprises at least one exogenous copy of a polynucleotide encoding MrgA protein comprising an amino acid sequence which is at least 95% identical to the amino acid sequence shown in SEQ ID NO: 2.

EVIDENCE APPENDIX

There is no evidence submitted under 37 CFR 1.130, 1.131 or 1.132 or any other evidence entered by the examiner and relied upon by the appellant in the appeal.

RELATED PROCEEDINGS APPENDIX

There are no decisions or pending related appeals and interferences per 37 CFR 41.37(c))(1)(x).